

Protocol for Real-Time RT-PCR assay with Lyoph P&P WNV Duo (adapted to TaqMan platform)

For RUO (Research Use Only)

Vial content

Lyoph P&P WNV Duo contains lyophilized primers and probe (P&P) for detection of West Nile virus (WNV). It must be used in combination with a generic RT-qPCR kit.

NB: Probe: FAM-QSY

Design

RT-qPCR systems included in Lyoph P&P WNV Duo:

- Detection of West Nile virus lineages 1 and 2 by real-time PCR. Linke et al., J Virol Methods, 2007. [DOI: 10.1016/j.jviromet.2007.05.021](https://doi.org/10.1016/j.jviromet.2007.05.021)
- Highly sensitive TaqMan RT-PCR assay for detection and quantification of both lineages of West Nilevirus RNA. Tang et al., J Clin Virol, 2006. [DOI: 10.1016/j.jcv.2006.02.008](https://doi.org/10.1016/j.jcv.2006.02.008)

Caution

Vials containing primers and probe mix (Lyoph P&P) must be stored at -20°C in the dark after reception. Stable 4 years under the described conditions.

Instructions

This protocol is adapted to the following kit and RT-qPCR platform:

- SuperScript™ III Platinum™ One-Step qRT-PCR Kit (ThermoFisher)
- CFX (BIORAD) RT-qPCR thermal cycler

Use of other kits or RT-qPCR platforms may require adaptation of the protocol.

I. Rehydration of Lyoph-P&P

- Write the date on the vial before opening
- Resuspend Lyoph P&P as follows (**these steps are critical to ensure adequate homogenization**):
 - ✓ Add nuclease-free water or RE buffer as described in Table 1
 - ✓ Homogenize by pipetting 10 to 20 times the volume up and down in the glass vial
 - ✓ Incubate rehydrated P&P at room temperature for 10 min
 - ✓ Perform a second series of 10-times pipetting.

Table 1. Lyoph-P&P regeneration; the number of tests/vial is indicated on the Lyoph P&P vial label.

Packaging (number of tests/vial)	8+1	16+2	24+2	32+3	48+3	96+5
H ₂ O or RE buffer (μL)	63	126	182	245	357	707

II. Preparation of the PCR reaction mix (for SuperScript™ III Platinum™ One-Step qRT-PCR Kit - ThermoFisher)

Table 2. Preparation of the PCR mix. *Rehydration performed as indicated in Table 1.

	Volume/PCR test (μL)
2X Reaction mix	12.5
Rehydrated Lyoph P&P*	7
SSIII/Taq enzyme mix	0.5
Total (1 PCR test)	20
Template RNA (H ₂ O for Ctrl-)	5
Final volume	25

III. Cycling program and RT-qPCR (for SuperScript™ III Platinum™ One-Step qRT-PCR Kit - ThermoFisher)

50°C	15min	45 cycles
95°C	2min	
95°C	15sec	
60°C	45sec (plate read)	

Read: FAM-QSY

Lyoph-P&P can be aliquoted and stored at -20°C. Each aliquot can be thawed only once and then discarded.

Caution: The WNV Duo assay amplifies a region of the 3'UTR that is well conserved between Flaviviruses, and in particular between WNV and Usutu virus (USUV), which are phylogenetically very close. The WNV Duo system can also detect Usutu virus; it is recommended to confirm positives with a specific Usutu system.